Assessment the impact of 17α-methyltestosterone hormone on growth, hormone concentration, molecular and histopathological changes in muscles and testis of Nile tilapia; *Oreochromis niloticus*

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Abstract: The present study was designed to explain clearly why methyltestosterone is widely used by the Egyptian producers of farmed tilapia and also to investigate its fate in treated fish to guarantee that no hazards on consumers, producers and on the environment. In this study, samples of untreated and treated Nile tilapia were collected at several time intervals. Water quality parameters were within the acceptable range for fish growth. The present analyses showed highly significant increase in body weight, body length, condition factor (K), HSI and GSI, between different time intervals (April - November, 2009) in the untreated control and treated groups. On the other hand, plasma testosterone and residual concentration of testosterone hormone in muscle showed highly significant differences between the studied months in untreated control and treated groups. Molecular biological analyses revealed that methyltestosterone was able to induce DNA fragmentation and molecular genetic variability (using RAPD- PCR fingerprinting pattern) in the testis tissues of the treated Nile tilapia; *Oreochromis niloticus*, which was higher in the first four studied months than the untreated control tilapia. Additionally, histopathological examination showed no changes and no traces of hormone accumulation in the muscle structure. Testis showed moderate number of spermatozoa followed by increasing in number of spermatozoa at the end of the study.
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1. Introduction

Tilapia species constitute a major and important item in the Egyptian fish farming. Tilapias are among the important fishes for aquaculture because of many positive characteristics and have been cultured in more than 100 countries (Altun *et al*., 2006).

The sex of fish can be significant in aquaculture because of differences between males and females in growth rate, size, behavior patterns, and breeding time. Administration of exogenous steroids can be effective in controlling sexual development (Al-ablani and Phelps, 2002). The synthetic steroid 17α- methyltestosterone is a male-specific hormone commonly used to induce sex reversal in teleost fish.

17α-Methyltestosterone (MT) is a synthetically produced anabolic and androgenic steroid hormone; i.e. it promotes both muscle growth and the development of male sexual characters. Hanson *et al*. (1984) reported that 10- 60 MT-treatment showed the best growth than control. On the other hand, Dan and Little (2000) who compared the culture performance of different species of stains of *O. niloticus* found that, MT treatment resulted a final size of fish 10.7 % larger than the mixed sex fish.

In a previous study, different doses of 17 α–methyltestosterone hormone (MT) used as a growth promoter was administrated to Nile tilapia; *Oreochromis niloticus* (L.) in fishmeal based pelleted diet for 90 days (Ahmad *et al*., 2002). The applied doses were 0.5, 1.0, 2.5, 5, 10, 20 and 40 mg MT/kg feed. The obtained results showed that only the dose of 5 mg/kg was the optimum effective dose in promoting significant final weight, weight gain and SGR of Nile tilapia (Ahmad *et al*., 2002).

In addition, Marjani *et al*. (2009) studied the effect of different doses of synthetic androgen 17α-Methyl Testosterone (MT), i.e., 50, 75 and 100 mg of hormone per kg of feed, on sex reversal and growth performance of Mozambique tilapia. They found that,

the dose rate 75mg/kg MT feed gave the maximum gain in body weight, i.e. 11.8 g, which is 1.2 times greater than the control.

On the other hands, Curtis *et al.* (1991) and Ahmad *et al*. (2002) found that plasma testosterone concentration is rapidly metabolized and excreted. Also, Rizkalla *et al*. (2004) found that whole body samples of normal fish and those treated for 28 days with 17 α-methyltestosterone (17 α-MT) contained detectable amounts of testosterone only in the first five months after the termination of feeding. Moreover, Rizkalla *et al*. (2004) found that, muscle samples taken from the monosex fish at marketable size, did not differ from the untreated controls and testosterone concentrations were below the detectable level (3ng/g).

Due to the misuse of hormonal treatments in sex reversal of tilapia especially in the Egyptian private sector hatcheries, the main objective of this study is to evaluate human food safety associated with sex reversal of Tilapia and may provide supporting data to approve the use of this hormone in aquaculture.

So, the present study aims to evaluate the effect of MT and its environmental impacts on the Nile tilapia in several aspects: (a) study its effect on the sex reversal ratio; (b) assessment its role as growth promoter on growth performance; (c) determination its concentration in the blood and the different vital organs ; (d) study its effect on the histological alterations; and (c) evaluation of its effect on the DNA damage which lead to abnormal changes of DNA fingerprints.

2. Materials and Methods

Fish

Nile tilapia was collected from World Fish Center Farm (WFC) in El- Abbassa, El-Sharkeya governorate and El- Nubaria fish farm, El-Behera governorate Egypt.

Experimental design and fish and water sampling

The present work was carried out on two groups of Nile tilapia fish; *Oreochromis niloticus* collected from natural condition and controlled farms, respectively a follows: (a) First group: (untreated Control tilapia growing in natural condition away from the hormonal effect) was taken from World Fish Center Farm (WFC) in El- Abbassa, El-Sharkeya governorate. Nile tilapia in this group ranged in length between 10.80 ± 0.26 cm and 22.77± 0.49 cm and ranged in weight between 19.2± 1.1 g and 256.7 ± 12.9 g. (b) second group (treated group): samples of tilapia fish were taken from El- Nubaria fish farm belongs to National Research Center (NRC) farm which is previously used the oral administration of the synthetic androgen (17 α-Methyl testosterone) hormone to produce all male tilapia at 60 mg/kg feed to newly hatched tilapia fry (9-11mm total length) for a period of 28 days which results in populations comprising 97 to 100% phenotype males (Popma and Green, 1990). This group was with range length between (11.53± 0.23 cm and 25.93 ± 0.78 cm) and range weight between was (25.4 ± 1.9 g and 287.4 ± 10.5 g). Samples of fish were taken through different months (April till November 2009) and also water samples will be taken to analyze all possible parameters to know the water quality using in the two sources and its contents.

Water samples, collected from the different studied sites, were analyzed for water temperature, oxygen content, pH, water hardness, total alkalinity, ammonia, nitrite and electric conductivity according to the method described by Association standard methods American Public Health (APHA, 1995).

Growth indices:

Body weight was recorded to the nearest gram and the total body length was measured to the nearest 0.1 cm for Nile tilapia; *Oreochromis niloticus* collected from the different studied sites.

a) Relative growth (RG) and Relative growth rate (RGR) (Busacker *et al*., 1990)**:**

Relative growth (%RG) = $[(W_2-W_1)/ W_1]$ x 100 (% / fish)

Relative growth rate (%RGR) = $[(W_2 –W_1) / (W_1$ x T)] x 100 (% / day)

Where W_1 : Initial weight at the start of the studied period (g).

W_2 : Final weight at the end of the studied period (g).

T : Time of the studied period.

b) Specific growth rate calculations (SGR) (Ahmad *et al*., 2002)**:**

Specific growth rate (SGR) = (ln W_2 – ln W_1) / T x 100 (% / day)

Where W_1 and W_2 are the initial and final weight, respectively, and T is the number of days of the feeding period.

c) Condition factor (k) (Schreck and Moyle, 1990):

K = (W / L^3) X 100

Where W : is the wet weight in g.

L : is the total length in cm.

d) Hepatosomatic index (HSI) (Schreck and Moyle, 1990):

H S I = (Weight of the liver / Total fish weight) X 100

e) Gonadosomatic index (GSI) (Abbas *et al*., 2008)**:**

G S I = (Weight of the gonads / Total fish weight) X 100

Tissue sampling:

Fish were dissected to get male testis and muscles which were kept frozen (- 20 ^{0}C) for determination of residual testosterone in muscle and DNA analyses of the Nile tilapia; *Oreochromis niloticus* collected from the different studied sites.

Determination of plasma testosterone and residual concentration of testosterone in muscle by Coat – A-Count Total Testosterone, Radioimmunoassay procedure:

The Coat-A-Count procedure is a solid-phase radioimmunoassay (RIA), based on testosterone- specific antibody immobilized to the wall of a polypropylene tube. ^{125}l-lable testosterone competes for a fixed time with testosterone in the sample for antibody sites. The tube is then decanted, to separate bound from free, and counted in a gamma counter. The amount of testosterone present in the sample is determined from a calibration curve.

Molecular biological analyses:

I- Quantitative analysis of DNA fragmentation

1-1 Diphenylamine reaction procedure

According to Burton (1956) testis tissues were used to determine the quantitative profile of the DNA fragmentation. The DNA fragmentation was determined in the pellets (P) and the supernatants of the samples. The proportion of fragmented DNA was calculated from absorbance reading at 600 nm wave length using the formula:

$$\%\text{Fragmented DNA} = \frac{OD(S)}{OD(S) + OD(P)} X\ 100$$

1-2 DNA gel electrophoresis laddering assay

Apoptotic DNA fragmentation was qualitatively analyzed by detecting the laddering pattern of nuclear DNA as described by Lu *et al*., (2002).

2. Random Amplification of Polymorphic DNA (RAPD-PCR) analysis

The genomic DNA was isolated using phenol/chloroform extraction and ethanol precipitation method with minor modifications (Sambrook *et al.*, 1989).

To generate RAPD profiles from the rat DNA, two random primer kits (A and C) from Operon Technologies (Operon, Almeda, CA, USA) were used. DNA amplification reactions were performed under conditions reported by Williams *et al*. (1990) and Plotsky *et al*. (1995). PCR amplification was conducted in 50 µl reaction volume containing 100 ng genomic DNA, 100 µM dNTPs, 40 nM primer, 2.5 units of Taq DNA polymerase and 5 µl promega 10X Taq DNA polymearse buffer.

Histopathological Examination:

Muscle and testis of Nile tilapia; *Oreochromis niloticus* , collected from different studied sites were fixed in neutralized formalin, dehydrated, embedded in paraffin wax and sectioned at 5 µm then stained with Haematoxylin and Eosin according to Carleton *et al*. (1967). Testes were classified by developmental stage based on histological criteria adapted from Grier (1981) and Bancroft *et al*., (1996).

Statistical analyses:

The results were statistically analyzed using Duncan's multiple range tests to determine difference in means Statistical Analyses System (SAS, 2000) and Software Program of Statistical Analysis (SPSS, 2008). One way ANOVA test (Analysis of variance) comparing the treated and untreated control groups in all months. Differences in all the studied parameters were assessed by one way ANOVA.

3. Results and Discussion

There is growing attention being given to the impact of pharmaceutically active compounds, including hormones released into the environment via wastewater discharge (Heberer, 2002). Anabolic steroids are potentially useful compounds in aquaculture due to their ability to increase weight gains and muscle deposition of treated fish.

In Egypt, there is a considerable interest in extending the culture of the Nile tilapia; *Oreochromis niloticus*, which gives a good quality fish with a high marketability and excellent growth rates (Kheir *et al*., 1998). Hanson *et al*. (1984) reported that 10- 60 ppm methyltestosterone treatment showed the best growth than control, these are also in line with Dan and Little (2000), who compared the culture performance of different strains of *Oreochromis niloticus* and found that considering all strains, MT treatment resulted in a final size of fish 10.7% larger than mixed sex fish.

Oral administration of the synthetic androgen 17 α-Methyltestosterone at 60 mg/kg feed to newly hatched tilapia fry (9-11mm total length) for a period of 28 days results in populations comprising 97 to 100% phenotype males (Popma and Green, 1990). Romerio *et al*. (2000) obtained 98% male population in *Oreochromis sp*., at dose rate of 60 mg kg-1 MT of feed. The results of this study showed a significantly lower male proportion (84.3%) for highest dose rate of MT (100 mg kg-1 of feed). These results are in line with the findings of Okoko (1996), who obtained 71.9% males at the dose rate of 120 mg kg-1 MT of feed.

The water quality of the aquatic habitat is considered the main factor controlling the state of health and disease in both cultured and wild fish. Nowadays, deterioration of the natural water resources conditions by the action of effluents discharged from various industries affect the quality and quantity of the fish (Elghobashy *et al*., 2001 and Zaghloul *et al*., 2005). In the current study, physicochemical properties of the water samples from the locations of the different sites were normal and there has been no change during the studied period (Table 1). There is no any negative impact on health and growth of fish naturally in the sampling areas of untreated control and treated fish during the studied period. The present results of water quality in El- Abbassa and El- Nubaria (Table 1) were within the acceptable range for fish growth (Boyd, 1984) and in agreement with Abdel-Tawwab and Ahmad (2009), who found that dissolved oxygen concentrations ranged from 6.6 to 7.4 mg L-1, the ambient water temperature range was 24.5-26.8 ºC, the pH range was 7.8-8.1, unionized ammonia concentration ranged from 0.11 to 0.19 mg L-1 and total alkalinity and total hardness ranges were 250-285 and 235- 290 mg L-1 as CaCO3 respectively.

Table (1) Water Quality Criteria for Water Samples collected from each fish farm for untreated control and treated sampling sites with 17 α methyl testosterone hormone of the Nile tilapia fish; *Oreochromis niloticus,* during April till November (2009).

Parameters	**Water quality for untreated control Site (El-Abbassa)**	**Water quality for treated site (El-Nubaria)**
Temperature (^{0}C)	(19-24) 22.00 ± 0.68	(19-27) 23.3 ± 1.23
Dissolved Oxygen (mg/L)	(6.11-8.2) 6.86 ± 0.32	(5.8-7.3) 6.6 ± 0.24
pH	(6.82 – 7.8) 6.85 ± 0.26	(6.45-7.8) 7.3 ± 0.14
Ammonia (mg/L)	(1.1 – 1.4) 1.27 ± 0.05	(1.3- 2.4) 1.9 ± 0.14
Total Hardness (mg/L as CaCO3)	(111 – 132) 120.00 ± 3.24	(129 – 161) 147.2 ± 4.48
Total Alkalinity (mg/L as CaCO3)	(186 -206) 189.33 ± 4.10	(196 – 228) 216.8 ± 4.53

- Data are represented as means of six samples ± SE.
- Student's t-Test between the two groups of the same parameter in the two studied sites for the whole studied period.

The obtained results in the present study revealed that administration of 17 α–methyltestosterone (MT) induced significant increase in fish growth of treated Nile tilapia (Table 2). These results are in agreement with the previous studies (Woo *et al*., 1993; Satpathy *et al*., 1995; Sambhu and Jayaprakas, 1997; Ahmad *et al*., 2002). In addition, methyletestosterone has been reported to enhance growth of various fish species such as Nile tilapia; *Oreochromis niloticus* (Tayamen and Shelton, 1978). The increase in body weight gain (Table 2) may attribute to that androgenic steroids enhance the release of growth hormone from the pituitary somatotrops of fish and/or induce the feed digestion and absorption rate causing increase in body weight (Yamazaki, 1976).

The values of the condition factor "k" are estimated for comparative purposes to assess the impact of environmental alterations on fish performance (Clark and Fraser, 1983). Therefore, the fluctuation in "k" may reflect the health condition of the fish as well as their protein and lipid contents (Weatherley and Gill, 1983). In the present study, the condition factor of the Nile tilapia, *Oreochromis niloticus*, collected from the different studied sites (Table 3) showed a significant difference in k values of fish collected from the different studied sites throughout the studied period. Steroid treatments have increased the condition factor of some salmonids (Fagerlund and McBride, 1975& 1977; Saunders *et al*., 1977) suggesting the increase in the percentage muscle of the body (Ahmad *et al*., 2002). The present results were also nearly similar with the

results which obtained by Winfree and Stickney (1981) who found the K values ranged from 1.8 to 2.85 in *Tilapia aurea*. The differences may be due to differences in season. We found that the significant gradual increase in (k) values of treated fish in September and that of untreated control fish collected in August and November may be due to the natural increase in the growth parameters of untreated control and treated fish as a result of natural growth during the studied period.

Hepatosomatic index (HSI) is another biological parameter that helps in studying growth of fish (Weatherley and Gill, 1987). Hepatosomatic index of the Nile tilapia; *Oreochromis niloticus* collected from the different studied sites (Table 3) showed progressive natural increase appeared in the untreated control and treated fish that amounted to a significant increase in the treated fish in the last three months of the study. This is in agreement with Ahmad *et al*. (2002) who found that, HSI was significantly changed at low MT doses (0.5, 1.0 and 2.5 mg MT/kg feed) and slightly increase at high MT doses (5, 10, 20 and 40 mg MT/kg feed).

Gonadosomatic index (GSI) of the Nile tilapia; *Oreochromis niloticus* collected from the different studied sites (Table 3) showed a progressive natural increase in the untreated control and treated fish, which amounted to a significant increase in the treated fish in the last month of the study (marketing season) and this is may be due to the effect of using 17α-methyltestosteronehormone.

The effect of 17α-methyltestosterone on the gonads appears to be complex (Ahmad *et al*., 2002). Macintosh *et al*. (1988) showed the higher level of 17-alpha methyltestosterone (60 mg/Kg of feed) produced some testicular degeneration which lowered the GSI value. Ahmad *et al*. (2002) found that male and female GSI was significantly decreased at high MT doses (5, 10, 20 and 40 mg MT/kg feed), while non-significant change were observed at low MT doses (0.5, 1.0 and 2.5 mg MT/kg feed).

Table (2): Relative growth rate (RGR) and specific growth rate (SGR) for untreated control and treated samples with 17 α- methyl testosterone hormone of the Nile tilapia fish; *Oreochromis niloticus,* collected from El- Abbassa and El-Nubaria fish farms during April till November (2009).

Parameter / Months	Mean weight (g) of Untreated control samples (El- Abbassa)	Mean weight (g) of treated samples (El- Nubaria)
April (Initial Mean)	19.2 ±1.1	20.1 ± 0.1
November (Final Mean)	256.7±12.9 **	287.4 ±10.5 **
Weight gain (g)	237.5	267.3
Relative growth (%RG)	206 % per fish	222 % per fish
Relative growth rate (%RGR)	5.2 % per day	5.5 % per day
Specific growth rate (SGR)	1.08 % per day	1.11 % per day

** Highly significant difference at P<0.01 between initial and final weight.

Table (3): Condition factor (k) , Hepatosomatic index (HSI) and Gonadosomatic index (GSI) for untreated control and treated samples with 17 α- methyl testosterone hormone of the Nile tilapia fish; *Oreochromis niloticus,* collected from El- Abbassa and El- Nubaria fish farms during April till November (2009).

Parameter / Months	(K) in control samples (El- Abbassa)	(K) in treated samples (El- Nubaria)	(HSI) in control samples (El-Abbassa)	(HSI) in treated samples (El- Nubaria)	(GSI) in control Samples (El- Abbassa)	(GSI) in Treated Samples (El- Nubaria)
April	1.52 ±0.05 ^{c}	1.64 ±0.05 ^{cd}	0.64 ±0.16 ^{c}	0.74 ±0.03^{b} *	0.16±0.01 ^{b}	0.17±0.03 ^{b}
May	1.88 ±0.10 ^{b}	1.55 ±0.08 ^{d} *	1.16 ±0.15 ^{b}	0.69 ±0.10 ^{b} **	0.19±0.01 ^{b}	0.1±0.02 ^{b}
June	1.86 ±0.06 ^{b}	1.69 ±0.06 ^{cd}	1.67 ±0.15 ^{b}	0.97 ±0.08 ^{b} **	0.17±0.02 ^{b}	0.13±0.01 ^{b}
July	1.99 ±0.06 ^{ab}	1.93 ±0.03 ^{bc}	1.71 ±0.25 ^{b}	0.83 ±0.12 ^{b} **	0.19±0.01^{b}	0.13±0.01^{b}
August	2.18 ±0.05 ^{a}	1.78 ±0.08 ^{bcd} **	1.51 ±0.15 ^{b}	0.59 ±0.04 ^{b} **	0.25±0.07 ^{b}	0.14 ±0.01^{b} **
September	1.98 ±0.05 ^{ab}	2.28 ±0.13 ^{a}	1.0 ±0.18 ^{bc}	2.44 ±0.36^{a} **	0.33±0.05 ^{c}	0.18±0.02^{b}
October	1.99 ±0.05 ^{ab}	2.03 ±0.08 ^{ab}	1.88 ±0.31 ^{bc}	2.50 ±0.32 ^{a} **	0.41±0.05 ^{b}	0.28 ±0.05 ^{c} **
November	2.17 ±0.10 ^{a}	1.68 ±0.14 ^{cd} *	2.34 ±0.23 ^{a}	2.44 ±0.31 ^{a}	0.67±0.14 ^{a}	1.01±0.45 ^{a}
F- Values	8.093**	7.057**	4.583**	15.896**	6.788**	3.594**

Data are represented as means of six samples ± SE.
Means with the same letter for each parameter in the same column between all months are non-significant different (P > 0.05); otherwise they do (SAS, 2000).
Student's t-Test between the two groups in the same month for the whole studied period.
One way ANOVA test (F-value) between all months in each group separately for the whole studied period.
* Significant difference at P<0.05 ** Highly significant difference at P<0.01.

Regarding to the concentration of the plasma testosterone hormone of the Nile tilapia collected from the different studied sites (Table 4) there is a significance difference in the concentrations of the untreated control and treated fish. There is a significant increase in the hormone concentrations of untreated control fish during June and July. This is may be due to male pairing with several females during mating season which starts in April or May and continue into September and October depending on water temperature as well as on the length of lighting period. This result is in agreement with the examination of the histopathological section of testis in the present study through these studied months. However, in treated fish the hormone concentration was high only in the first month of treatment, then gradually decreased till the end of the studied period. The plasma testosterone concentration seems not existing (detectable amounts), which indicates that, there is no effect of the used hormone at the end of the study period. The rapid metabolism and excretion of MT by a fish treated early in its life history, combined with the extended period needed to produce a marketable size fish results in a safe consumer product (Phelps, 2001). Ahmad *et al*. (2002) also reported that digested MT is rapidly metabolized and excreted. These results are in accordance with Rizkalla *et al*. (2004), who reported that whole body samples of normal fish and those treated for 28 days with 17 α-methyltestosterone (17 α-MT) contained detectable amounts of testosterone only on the first five months after the termination of the feeding with 17 α- methyltestosterone.

Table (4) Plasma concentration of the testosterone hormone (ng/ml) and Residual concentration of testosterone in muscles (ng/g) for untreated control and treated samples with 17 α- methyl testosterone hormone of the Nile tilapia fish; *Oreochromis niloticus,* collected from El- Abbassa and El- Nubaria fish farms during April till November (2009).

Parameter / Months	Plasma testosterone in control samples (El- Abbassa)	Plasma testosterone in treated samples (El- Nubaria)	Residual testosterone in muscles in control samples (El- Abbassa)	Residual testosterone in muscles in treated samples (El- Nubaria)
April	7.16 ±0.58 ^{c}	16.42 ±0.71 ^{a} **	0.95 ±0.07 ^{e}	2.05 ±0.20 ^{ab} **
May	6.35 ±0.29 ^{cd}	7.26 ±0.59 ^{b}	0.64 ±0.04 ^{e}	0.86 ±0.03 ^{c} **
June	30.39 ±0.63 ^{a}	6.50 ±0.31 ^{bc} **	2.60 ±0.58 ^{cd}	1.78 ±0.29 ^{b}
July	20.64 ±0.86 ^{b}	5.31 ±0.36 ^{cd} **	4.09 ±0.60 ^{b}	1.91 ±0.13 ^{ab} **
August	4.94 ±0.32 ^{de}	5.55 ±0.35 ^{cd}	4.44 ±0.33 ^{b}	0.83 ±0.05 ^{c} **
September	3.42 ±0.76 ^{ef}	4.41 ±0.39 ^{d}	5.61 ±0.33 ^{a}	1.38 ±0.36 ^{bc} **
October	2.09 ±0.06 ^{fg}	2.56 ±0.31 ^{e}	3.63 ±0.32 ^{bc}	2.67 ±0.41 ^{a}
November	1.10 ±0.07 ^{g}	1.29 ±0.15 ^{e}	1.52 ±0.30 ^{ed}	1.44 ±0.33 ^{bc}
F-Values	** **381.804**	** **112.483**	** **22.762**	** **5.438**

- Data are represented as means of six samples ± SE.
- Means with the same letter for each parameter in the same column between all months are non- significant different (P > 0.05); otherwise they do (SAS, 2000).
- Student's t-Test between the two groups in the same month for the whole studied period.
- One way ANOVA test (F-value) between all months in each group separately for the whole studied period.
- * Significant difference at P<0.05 ** Highly significant difference at P<0.01.

It is apparent from the previous literatures that 17 α- methyltestosterone has an important economic potential for use in fish culture. However, before this synthetic steroid is used on a large scale in Egypt, and although there is a very long interval between treatment and harvest, it is necessary to investigate its fate in treated fish to guarantee that no hazards for consumer if residues remain in the destined for marketing as reported by Rizkalla *et al*. (2004). Literature regarding steroid residues in tissues of fish treated with androgenic steroids has been reviewed by Donaldson *et al*. (1979) and Higgs *et al*. (1982). They indicated that steroids are rapidly metabolized and /or excreted from fish tissues. 17 α-methyltestosterone was eliminated from tissues more slowly than testosterone (Fagerlund and McBride, 1978 and Fagerlund and Dye, 1979), and it was postulated that the presence of the 17 α- methyl group enhance efficacy of the synthetic steroid over endrogenous steroids (Donaldson *et al*., 1979). Hormone elimination is rapid in fish because it is believed to occur mainly via excretion in the faeces and via the gills (Cravedi *et al*., 1993).This rapid metabolism and excretion of methyltestosterone by a fish treated early in its life history, combined with the extended period needed to produce a marketable size fish results in a safe consumer product (Phelps, 2001).

In the current work, residual concentration of the testosterone hormone in muscles of the Nile tilapia collected from the different studied sites (Table 4), there is a significance difference in the residual concentration of testosterone hormone in muscles in the untreated and treated fish. The

presence of hormone residues is not high in muscle tissue of the untreated and treated fish and decreased in the last month of the study which is the marketing season for this fish. This indicates that there is no effect of the used hormone at the end of the studied period. These findings were in accordance with Johnstone *et al*. (1983), who found > 95% of the radio-labeled MT in the viscera and no radioactivity could be found 50 h post-treatment. They were also in agreement with Goudie *et al*. (1986), who reported that tilapia fry fed for 30 days a feed containing radioactive labeled methyltestosterone showed a rapid depletion of radioactive methyltestosterone from tilapia muscle with only a trace of methyltestosterone could be found.

El- Nemr *et al*. (1999) recorded that the residual value of 17 α- methyltestosterone in *Oreochromis niloticus* fry muscle was significantly dropped after 6 weeks withdrawal period and still higher than control group. Our results agree also with Rizkalla *et al*. (2004), who reported that muscle samples taken from the monosex fish did not differ from the untreated controls and testosterone concentrations were below the detectable level (3ng/g). Based on the scientific evidence that methyltestosterone is rapidly eliminated from fish, and therefore, there is no possibility that methyltestosterone will persist in adult fish after the several months required for farmed tilapias to reach marketable size.

The molecular genetic variability of the treated tilapia genomes and their control were evaluated using two primer kits (A and C). Only five of these primers (10-mer random primers: (A04: 5'-AAT CGG GCTG-3', A08: 5'-GTG ACG TAGG-3', A10: 5'-GTG ATC GCAG-3', C09: 5'-CTC ACC GTCC-3', C12): 5'-TGT CAT CCCC-3') gave positive and detectable bands (Fig. 3). PCR-based techniques, such as RAPDs, have previously allowed the discrimination as well as estimation of genetic variation attributed to genotoxic elements. The exposure to genotoxic agents will give rise to alterations of DNA structure that can lead to abnormal changes of DNA fingerprints. Therefore, we have applied the random amplified polymorphism DNA (RAPD) method to evaluate the genotoxic effects.

Methyltestosterone usage in tilapia farming is expected to continue to increase rapidly as the global demand for large whole tilapia and tilapia fillets grows. Despite widespread use of the androgen 17α-methyltestosterone (MT) in tilapia farming, the implications of tilapia hormone treatment in relation to human health and the environment have not been well articulated to the fish trade or the general public.

The molecular biological results of the present study (Table 5 and Figs. 1, 2 &3) revealed that methyltestosterone was able to induce DNA fragmentation in testes of Nile tilapia in the first four studied months after MT treatment to induce sex reversal in farmed tilapias compared to the untreated control tilapia. In addition, the molecular genetic variability (using RAPD fingerprinting pattern) among the treated tilapia (in liver and testes tissues) was higher in the first four studied months after treatment than the untreated control tilapia.

For our knowledge, there are no data regarding the effect of methyltestosterone on the DNA damage in fish especially Nile tilapia. However, it could be postulated that the methyltestosterone residues were still existed in the fish tissues and/or in the fish environment up to the first four months after treatment and then began to be disappeared, whereas the DNA fragmentation decreased after the first four studied months.

The action mechanism of testosterone treatment inducing genetic toxicity during the first months of age in tilapia (tilapia fry) is not investigated yet. In the present study, the negative effect of testosterone induced DNA damage may be attributed to the weakness in the immune system which may not be completed in growth yet. The main way in which steroid hormones interact with cells is by binding to proteins called steroid receptors. When steroids bind to these receptors, the proteins move into the cell nucleus and either alter the expression of genes (Lavery and McEwan, 2005) or activate processes that send signals to other parts of the cell (Cheskis, 2004) cause genetic toxicity. Beg *et al* (2008) reported that the possible genotoxicity of testosterone is depend on the metabolic activation.

The results of the present study (Figs. 1, 2 &3) revealed that the DNA damage attributed to methyltestosterone treatment was markedly disappeared after the first four studied months until it reached a relative stability rate similar to control untreated tilapia. It could be explained that the methyltestosterone residues in the fish tissues and/or in the fish environment were removed. Furthermore, disappearance of the DNA damage may be attributed to the increase of the immunity defense in the growing fish.

In agreement with our results, Hana *et al* (2008) reported that there were no significant differences in the frequency of total chromosomal aberrations between control and testosterone propionate-treated adult mice. In addition, they found that the molecular genetic variability using RAPD-PCR among the testosterone-treated adult mice was similar to control untreated mice. Whereas, all of the oligodecamers used revealed monomorphic bands in

the control samples and those treated with testosterone propionate.

Additionally, histopathological examination or biomarkers have been increasingly recognized as a valuable tool for field assessment of the impact of using 17α-methyltestosterone hormone on fish organs (Heath, 1995; Schwaiger *et al*., 1996 and Teh *et al*., 1997). The investigated biochemical and physiological changes were confirmed by histopathological alterations of muscle, liver and testis of the Nile tilapia collected from the two fish farms.

Table (5): Effect of the 17 α methyl testosterone hormone on the DNA fragmentation ratio in testis tissues collected from Nile tilapia; *Oreochromis niloticus,* for several time intervals (April - November, 2009).

Parameter / Months	DNA fragmentation % in testis tissue for control samples (El- Abbassa)	DNA fragmentation % in testis tissue for treated samples (El- Nubaria)
April	10.66 ±0.33^{a}	12.66 ±0.33^{a}
May	10.00 ±0.57^{a}	12.66 ±0.33^{a}
June	10.66 ±0.33^{a}	11.66 ±0.33^{ab}
July	10.33 ±0.33^{a}	11.66 ±0.33 ^{ab}
August	10.33 ±0.33^{a}	10.66 ±0.33^{b}
September	10.66 ±0.33^{a}	10.66 ±0.33^{b}
October	10.66 ±0.33^{a}	10.66 ±0.33^{b}
November	10.66 ±0.33^{a}	10.66 ±0.33^{b}
F-Values	6.843 **	6.614 **

- Data are represented as means of six samples ± SE. Means with the same letter for each parameter in the same column between all months are non- significant different (P > 0.05); otherwise they do (SAS, 2000). Student's t-Test between the two groups in the same month for the whole studied period. One way ANOVA test (F-value) between all months in each group separately for the whole studied period. * Significant difference at P<0.05 ** Highly significant difference at P<0.01.

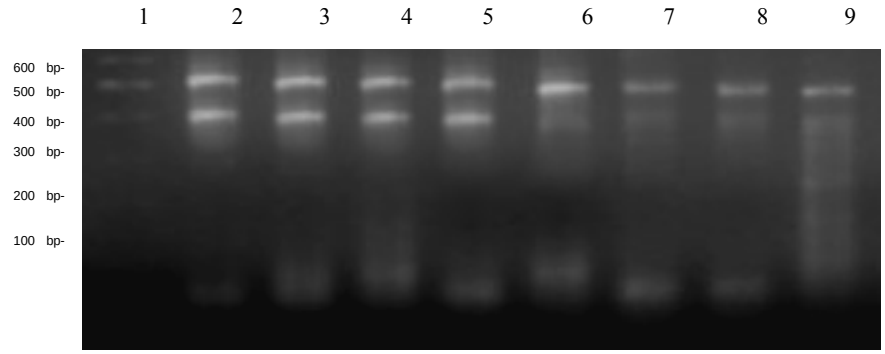

Figure (1): DNA fragmentation detected with agarose gel electrophoresis of tilapia DNA extracted from testis exposed to testosterone in different time intervals analyzed by DNA gel electrophoresis laddering assay. Lane 1 represents DNA ladder. Lanes 2 to 9 represent testis tissues collected from April to November (2009).

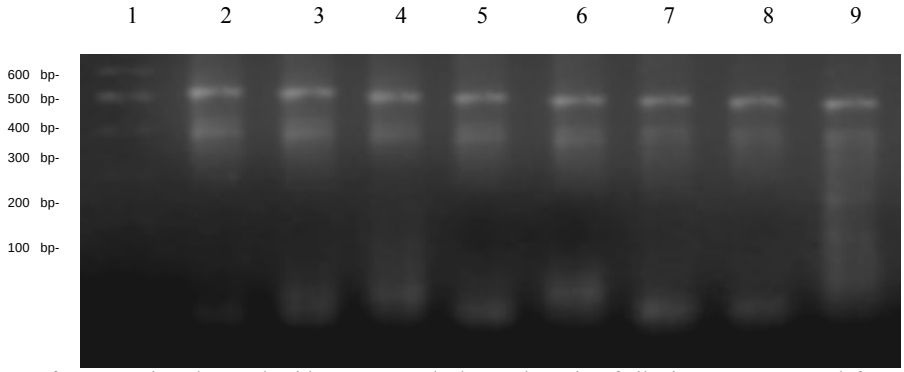

Figure (2): DNA fragmentation detected with agarose gel electrophoresis of tilapia DNA extracted from untreated testis in different time intervals analyzed by DNA gel electrophoresis laddering assay. Lane 1 represents DNA ladder. Lanes 2 to 9 represent testis tissues collected from April to November (2009).

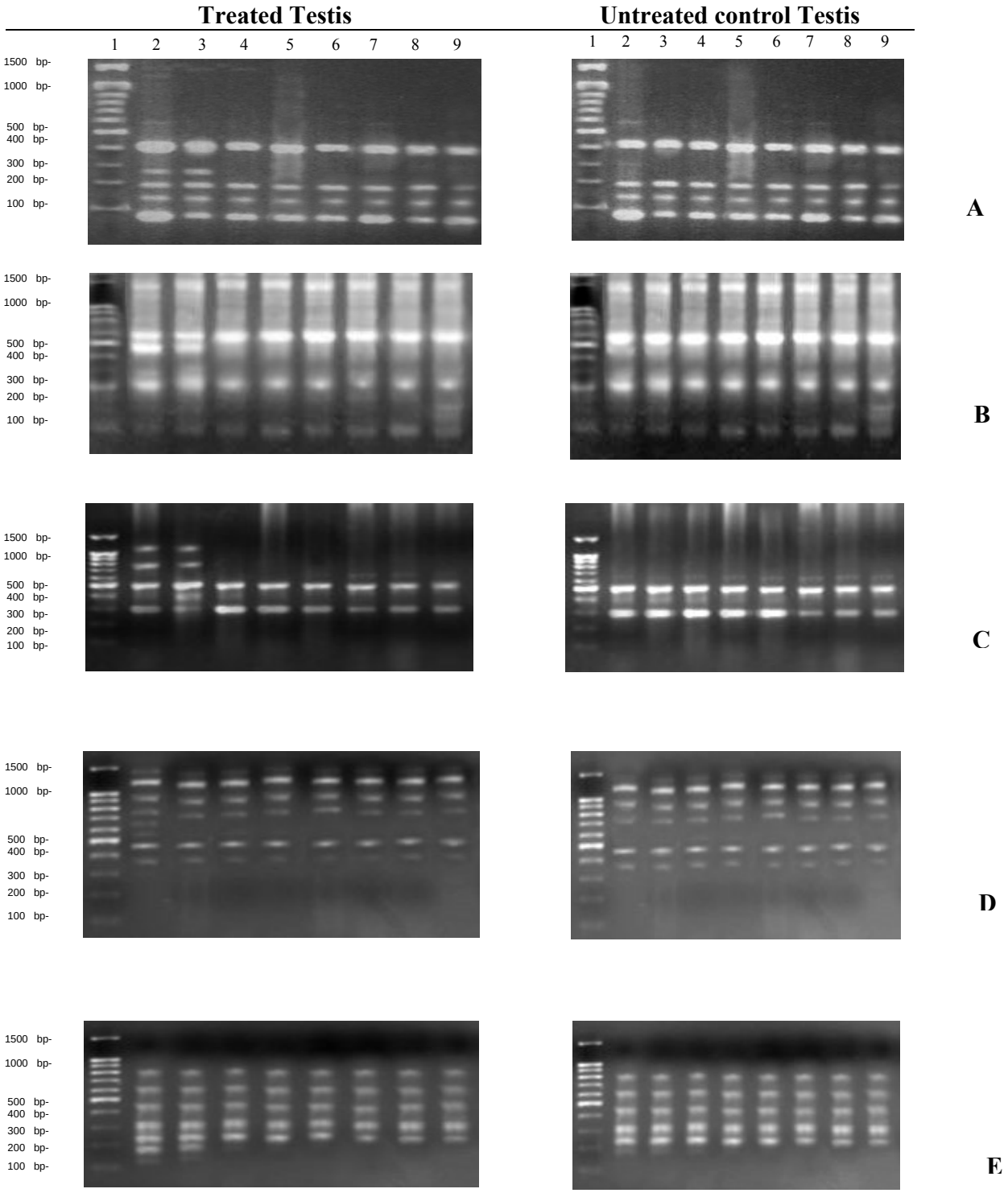

Figure (3). Comparison of RAPD fingerprinting profiles of different tilapia genomic DNA. (A) Represents PCR products with primer A04, (B) Represents PCR products with primer A08, (C) Represents PCR products with primer A10, (D) Represents PCR products with primer C09, (E) Represents PCR products with primer C12. The DNA marker is in lane 1. Lane 2 to Lane 9 represent months of collection (April till November, respectively) of fish testis tissue samples treated with 17 α- methyltestosterone throughout the period of study.

The histopathological examination revealed that muscles sections of control and treated fish showed normal structure throughout the experimental period (Photomicrograph 1). There was no change in the composition of the muscle, and also there were no traces of hormone accumulation in the muscle tissue of fish during the studied period. These results are in agreement with Curtis *et al*. (1991), who reported that digested methyltestosterone is rapidly metabolized and excreted. This rapid metabolism and excretion of methyltestosterone by a fish treated early in its life history combined with the extended period needed to produce a marketable size fish results in a safe consumer product (Phelps, 2001).

The histopathological finding showed that the effect of 17 ∝-methyltestosterone on the gonads appears to be complex. Testes shown to be bilobed with spermatogonia dispersed throughout the gametogenic epithelium of the seminiferous tubules. Each testicular lobe covered luminally by an epithelium consisting of primary germ cells and Sertoli cells. The highest number of spermatogonia was found at the apical ends of the tubules. During maturation extensions of Sertoli cells surround single or small groups of B-spermatogonia, forming the spermatocysts, the final dimensions of which reflect the final number of contained spermatozoids (Fishelson *et al*., 2006).

Regarding the control samples the histopathological examination showed moderate number of spermatozoa inside the seminiferous tubules and which followed by increasing in number of spermatozoa inside seminiferous tubules this appear especially in June and July (Photomicrograph 2). This confirmed by the present results of the plasma testosterone analysis.

Testis sections of treated fish collected from the different studied sites showed moderate number of spermatozoa inside seminiferous tubules which followed by high density of spermatozoa inside seminiferous tubules till the end of the studied period (Photomicrograph 2). Generally this increase reflects the enhancing effect of using 17 α-methyltestosterone hormone.

Our results were in agreement with Yamazaki (1972) in pink and chum salmon and by Hirose and Hibiya (1968a & 1968b) in goldfish and rainbow trout, who reported that MT administration of 2.5 mg/kg induced the degenerative changes in the ovaries and testes. Furthermore, Higgs *et al*. (1977) found clear signs of gonads degeneration in coho salmon affected by MT causing fish sterility, which might be considered advantageous in fish culture because less food energy would be channeled into gonodal development and therefore more would be available for body growth.

The above results demonstrated that 17α-methyltestosterone has an important economic potential for use in fish culture. Also, methyltestosterone treatment in tilapia farming is considered to be entirely safe provided the following recommended best practices are adopted by producers: (1) They restrict tilapia methyltestosterone treatment to the early fry stages. (2) They limit the dosage of methyltestosterone used to a maximum of 60 mg methyltestosterone /kg fry feed. (3) They rear methyltestosterone treated tilapia fry to adult size for at least five months after hormone treatment ends to ensure zero hormone residue remains in the fish. (4) As a precautionary measure, adopt safe handling protocols when preparing and administering methyltestosterone treated tilapia feed; use latex gloves and a protective face mask to avoid dermal contact or inhalation of methyltestosterone. (5) They keep a careful inventory of the amounts of methyltestosterone supplied to and used in each tilapia hatchery, and ensure that access of the hormone supply and record-keeping are controlled by the farm manager or hatchery supervisor. (6) They avoid direct release of hatchery water used for methyltestosterone treatment of tilapia fry into the environment. As a precautionary measure, tilapia hatcheries should utilize a gravel and sand filter, plus a shallow vegetated pond or an enclosed wetland, to receive and hold the hatchery wastewater for several days before discharge into the general environment.

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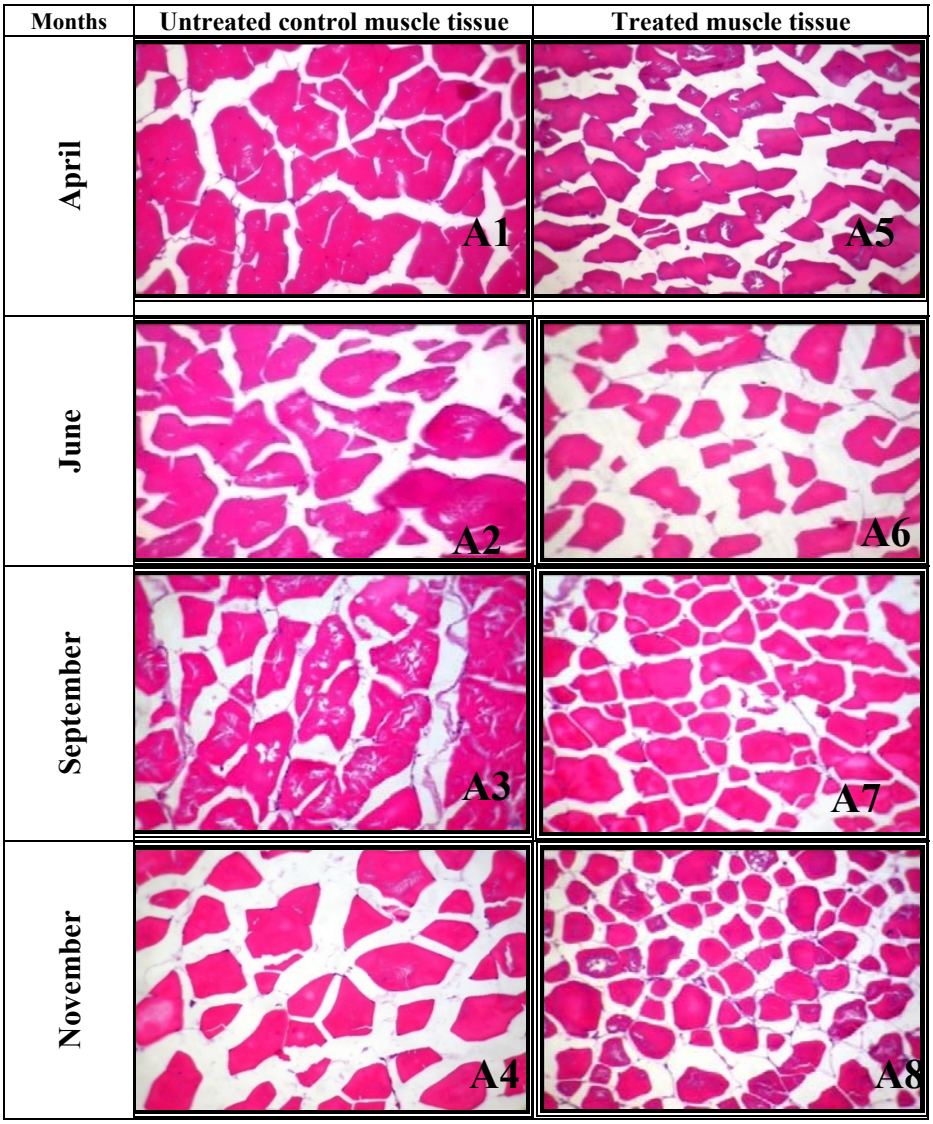
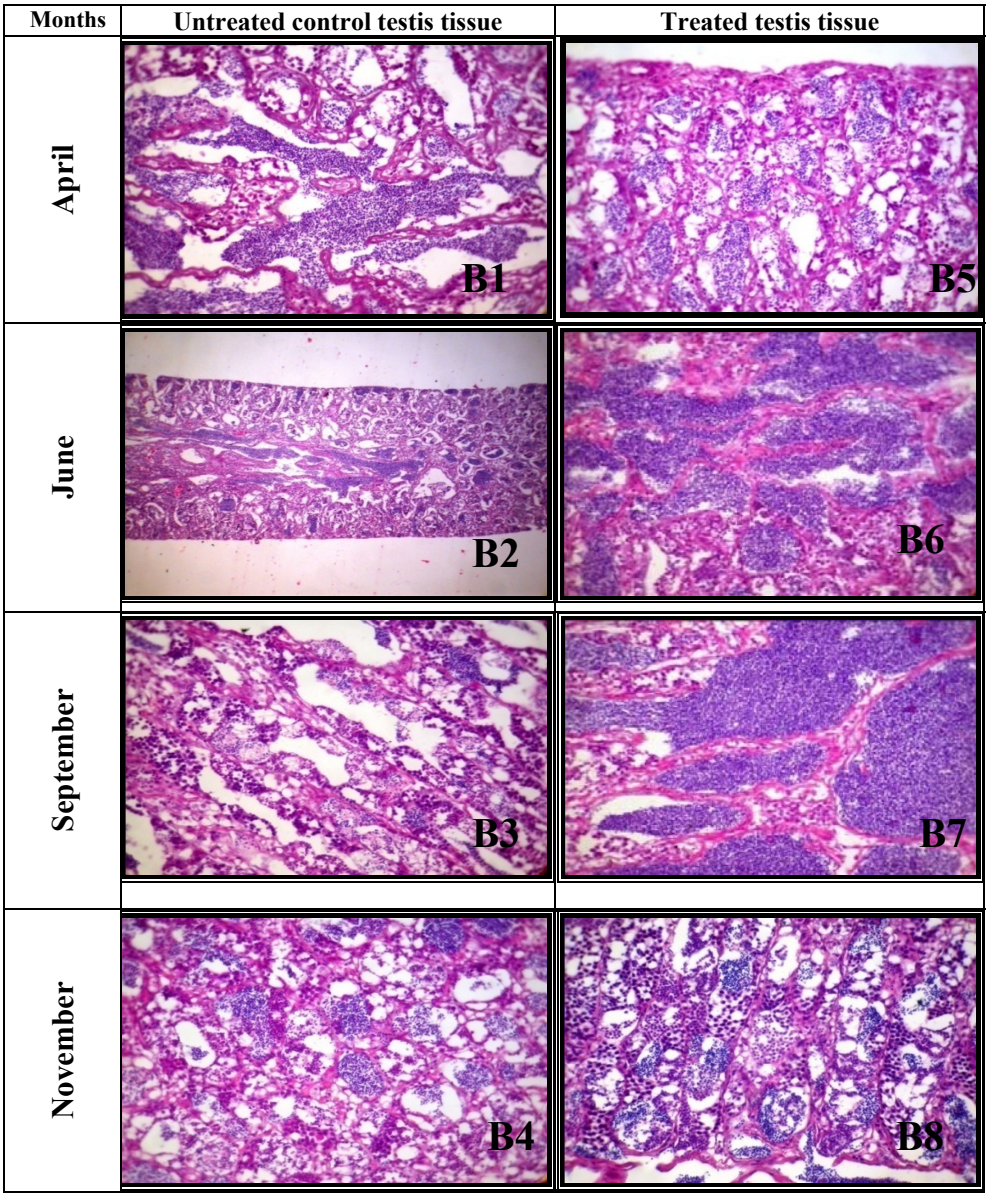


Photomicrograph (1):
Histological sections in skeletal muscle tissues of *Oreochromis niloticus* collected from the untreated control fish in El- Abbassa and treated fish in El-Nubaria fish farms from April till November (2009).

Untreated control
(A1) Skeletal muscles showing normal structure (H&E 400X).
(A2) Skeletal muscles showing normal structure (H&E 400X).
(A3) Skeletal muscles showing normal structure (H&E 400X).
(A4) Skeletal muscles showing normal structure (H&E 400X).

Treated
(A5) Skeletal muscles showing normal structure (H&E 400X).
(A6) Skeletal muscles showing normal structure (H&E 400X).
(A7) Skeletal muscles showing normal structure (H&E 400X).
(A8) Skeletal muscles showing normal structure (H&E 400X)



Photomicrograph (2): Histological sections in testis tissues of *Oreochromis niloticus* collected from the untreated control fish in El- Abbassa and treated fish in El-Nubaria fish farms from April till November (2009).

Untreated control
(B1) Testis showing moderate number of spermatozoa inside seminiferous tubules (H&E 400X).
(B2) Testis showing moderate number of spermatozoa inside seminiferous tubules (H&E 200X).
(B3) Testis showing moderate number of spermatozoa inside seminiferous tubules (H&E 400X).
(B4) Testis showing increased number of spermatozoa inside seminiferous tubules (H&E 400X).

Treated
(B5) Testis showing moderate number of spermatozoa inside seminiferous tubules (H&E 400X).
(B6) Testis showing high density of spermatozoa inside seminiferous tubules (H&E 400X).
(B7) Testis showing high density of spermatozoa inside seminiferous tubules (H&E 400X).
(B8) Testis showing high density of spermatozoa inside seminiferous tubules (H&E 400X).

References

Abbas, H. H.; Ali, F. K. and Kenawy, A. M. (2008): Assessment Study about Using Underground Water for Tilapia Culture for the First Time in El-Bahria Oasis desert, Egypt, Nutrition and Health., 19: 237–256.

Abdel-Tawwab, M. and Ahmad, M. H. (2009): Effect of dietary protein regime during the growing period on growth performance, feed utilization and whole-body chemical composition of Nile Tilapia; *Oreochromis niloticus* (L.) Aquacul. Res., 40: 1532-1537.

Ahmad, M.H.; Shalaby, A.M.E.; Khattab, Y.A.E. and Abdel-Tawwab, M. (2002): Effects of 17 α–methyltestosterone on growth performance and some physiological changes of Nile tilapia fingerlings (*Oreochromis niloticus* L.). Egypt. J. Aquat. Biol. Fish, 4(4): 295-311.

Al-ablani, S.A. and Phelps, R.P. (2002): Paradoxes in exogenous androgen treatments of bluegill. *J. Appl. Ichthyol.*, 18:61-64.

Altun,T. ; Tekelioğlu,N. and Danabaş,D.(2006): Tilapia Culture and Its Problems in Turkey. E.U. J. Fish.Aquat. Sci., 23 (3-4): 473–478.

American Public Health Association (APHA), American Water Works Association and Water Pollution Control Federation (1995): Standard Methods for the Examination of Water and Wastewater. 19th edition, New York, N.Y.

Bancroft, D.; Stevens, A. and Turner, R. (1996): Theory and Practice of Histological Techniques, 4th edn. Churchill Livingstone: Edinburgh,London, Melbourne.

Beg, T.; Siddique, Y. H.; Ara, G. ; Afzal, M. and Gupta, J. (2008): Antioxidant Effect of ECG on Testosterone Propionate Induced Chromosome Damage. Human Genetics and Toxicology Laboratory, Department of Zoology, Aligarh Muslim University, Aligarh 202002 (UP), India.Interna.J.Phamacol. 4 (4): 258-263.

Boyd, C.E. (1984): Water Quality in Warm water Fishponds. Auburn University, Agricultural Experimental Station, Auburn, AL, USA.

Burton, K. (1956): The study of the conditions and mechanisms of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem. J., 62: 615–623.

Busacker, G.P.; Adelman, I.R. and Goolish, E.M. (1990): Growth. In: Methods for Fish Biology. Schreck, C.B. and P.B. Moyle (Eds.), American Fisheries Society, USA., pp: 363-387.

Carleton, M.; Drary, Y.; Wallington, E.A. and Cammeron, H. (1967): Carleton's Histological Technique, 4th ed., Oxford Univ. Press, New York, Toronto.

Cheskis, B. (2004): Regulation of cell signaling cascades by steroid hormones. J. Cellular Biochem., 93 (1): 20–7.

Clark, E.R. and Fraser, J.A.L. (1983): The survival and growth of six species of freshwater fish, in tap water and diluted and undiluted effluents from sewage percolating filters.J.Fish Biol., (22): 431-445.

Cravedi, J.P.; Delous, G.; Debrauwer, L. and prome, D. (1993): Biotransformation and branchial excretion of 17-alpha methytestosterone in trout. Drug Meta.Disposition, 21: 377-385.

Curtis, L.R.; Diren, F.T.; Hurley, M.D.; Seimand, W.K. and Tubb, R.A. (1991): Disposition and elimination of 17 ∝-testosterone in Nile tilapia (*Oreochromis niloticus*). Aquacul., 99: 193-201.

Dan, N.C. and Little, D.C. (2000): The culture performance of mono-sex and mixed-sex new season and overwintered fry in three strains of *Nile tilapia in* North Vietnam. Aquacul., 184: 221-231.

Donaldson, E.M.; Fagerlund, U.H.M.; Higgs, D.A. and McBride, J.R. (1979): Hormonal enhancement of gorwth in fish In: "Fish Physiology", Vol. 8, pp. 455-598 Edited by W.S. Hoar. D.J. Randall and J.R. Brett Academic Press Inc, New York and London.

Elghobashy, A.H.; Zaghloul, K.H. and Metwally, M.A. (2001): Effect of some water pollutants on the Nile tilapia; *Oreochromis niloticus* collected from the river Nile and some Egyptian Lakes.Egypt.J.Aquat. Biol. Fish., 5(4): 251- 279.

El-Nemr, I.Z; Haleem, H.H. and Abdou, K.A.H. (1999): Evaluation of the effect of 17 α-methyltestosterone on monosex, mortality rate and residual values in tilapia (*Oreochromis niloticus*). Beni- Suef Vet.Med. J., 9(2):25-33.

Fagerlund, U.H.M. and McBride, J.R. (1975): Growth increments and some flesh characteristics of juvenile coho salmon receiving diets supplemented with 17 α-methyltestosterone. J. Fish Biol., 7: 305-314.

Fagerlund, U.H.M. and McBride, J.R. (1977): Effect of 17 α-methyltestosterone on growth, gonad development, external features and proximate composition of muscle of steelhead trout, coho and pink salmon. Fish. Mar. Serv. Tech. Rep., 716: 36-39.

Fagerlund, U.H.M. and McBride, J.R. (1978): Distribution and disappearance radioactivity in blood and tissues of coho salmon (*Oncrhynchus kisutchi*) after oral administration of ^{3}H-testosterone. J.Fish Res. Bd. Can., 35: 893-900.

Fagerlund, U.H.M.; Dye, H.M. (1979): Depletion of radioactivity from yearling coho salmon (*Oncrhynchus kisutchi)* after extended ingestion of anabolically effective doses of 17a-methyltestosterone-1,2-^{3}H. Aquacul., 18: 303–315.

Fishelson, L.; Delarea, Y. and Gon, O. (2006): Testis structure, spermatogenesis, spermatocytogenesis, and sperm structure in cardinal fish (Apogonidae, Perciformes). Department of Zoology and Electronmicroscopy Unit, George S. Wise Faculty of Life Sciences, Tel Aviv University, 69978 Tel Aviv, Israel. fishelv@post.tau.ac.il. Anat Embryol (Berl). 211(1):31-46.

Goudie, C.A.; Shelton, W.L. and Parker, N.C. (1986): Tissue distribution and elimination of radiolabelled methyltestosterone fed to sexually undifferentiated blue tilapia. Aquacul., 58: 215-226.

Grier, H.J. (1981): Cellular organization of the testis and spermatogenesis in fishes. *Am.Zool.*, 21:345-357.

Hana, Y.H.; Khalil, W. K.B.; Elmakawy, A. I. and Elmegeed, G. A. (2008): Androgenic profile and genotoxicity evaluation of testosterone propionate and novel synthesized heterocyclic steroids. J. Steroid Biochem. Mol. Biol., 110: 284–294.

Hanson, R.; Sinith Evmanm, R.O.; Shelton, H.W. and Dunham, R.A. (1984): Growth comparison of monosex tilapia produced by separation of sexes hybridization and sex reversal. M.Sc. Thesis, Auburn University, pp: 570.

Heath, A.G. (1995): Water Pollution and Fish Physiology. CRC. Press. Inc. Boca Raton, Florida. 359pp.

Heberer, T. (2002): Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. *Toxicol. Lett.,* 131: 5–17.

Higgs, D.A.; Fagerlund, U.H.M.; Eales, J.G. and McBride, J.R.; (1982): Application of thyroid and steroid hormones as anabolic agents in fish culture. Comp.Biochem.Physiol., 73 B (1):143-176.

Higgs, D.A.; Fagerlund, U.H.M.; McBride, J.R.; Dye, H.M. and Donaldson, E.M. (1977): Influence of combinations of bovine growth hormone, 17 α-methyltestosterone and L-thyroxine on growth of young coho salmon (*Oncorhynchus kisutch*). Can. J. Zool., 55: 1048-1056.

Hirose, K. and Hibiya, T. (1968 a): Physiological studies on growth promoting effect of protein-anabolic steroids on fish. I. Effects on goldfish. Bull. Jap. Soc. Sci. Fish., 34: 466-472.

Hirose, K. and Hibiya, T. (1968 b): Physiological studies on growth promoting effect of protein-anabolic steroids on fish. I. Effects of 4-chlorotestosterone acetate on rainbow trout. Bull. Jap. Soc. Sci. Fish., 34: 473-481.

Johnstone, R.; Macintosh, D.J. and Wright, R.S., (1983): Elimination of orally administered 17α-Methyltestosterone by *Oreochromis mossambicus* (Tilapia) and Salmo gairdneri (rainbow trout) juveniles. Aquacul., 35: 249-257.

Kheir, M.T.; Mechail, M.M. and Abo-Hegab, S. (1998): Growth of adult and newly hatched fry of Oreochromis niloticus reared at different saline concentrations. Egypt. J. Zool., (30):107- 115.

Lavery, D.N. and McEwan, I.J. (2005): Structure and function of steroid receptor AF1 transactivation domains: induction of active conformation. Biochemical J., 391 (Pt 3): 449–64.

Lu, T.; Xu, Y. and Mericle, M.T. (2002): Quantitative analysis of DNA fragmentation. Biochimica. et Biophysica. Acta., 1590: 16-20.

Macintosh, D.J. and Vargheso, G.P.S. (1985): Rao hormonal sex reversal of wild spawned tilapia in India. J. Fish Biol., 28: 87-94.

Macintosh, D.J.; Singh,T.B.; Little, D.C. and Edwards, P.(1988): Growth and sexual development of 17 ∝-methyltestosterone and progesterone- treated Nile tilapia (*Oreochromis niloticus*) reared in earhen ponds. In R.S.V. pullin, T. Bhukaswan, K. Tongothai and J.L.Maclean (Editors), The second International Symposium on Tilapia in Aquaculture, Bangkok, Thailand,Manila, Philippines.pp. 457- 463.

Marjani, M.; Jamili, S.; Mostafavi, P.G.; Ramin, M. and Mashinchian, A. (2009): Influence of 17-alpha methyl testosterone on masculinization and growth in Tilapia (*Oreochromis mossambicus*). J. Fish. Aquat. Sci., 4(1): 71-74.

Okoko, M. (1996): Effect of 17-alpha methyltestosterone concentrations on the sex ratio and gonadal development of Nile tilapia; *Oreochromis niloticus*. M.Sc. Thesis. Auburn University, Alabama, US.

Phelps, R.P. (2001): Sex reversal: the directed control of gonodal development in tilapia. Pages 35-60. In: D.E. Meyer (ed.). Proceedings for Tilapia Sessions from the 6th Central American Aquaculture Symposium. 22-24 August 2001, Tegucigalpa, Honduras.

Plotsky, Y.; Kaiser, M.G. and Lamont, S.J. (1995): Genetic characterization of highly inbred chicken lines by two DNA methods: DNA fingerprinting and polymerase chain reaction using arbitrary primers. Animal Genetics, 26: 163-170.

Popma, T.J. and Green, B.W. (1990):. Sex reversal of tilapia in earthen ponds. International Center for Aquaculture and Aquatic Environments Research and Development Series No. 35, Auburn University, Alabama, pp: 15.

Rizkalla, E.H.; Haleem, H.H.; Abdel-Halim, A.M.M and Youssef, R.H. (2004): Evaluation of using 17α-methyl testosterone for monosex *Oreochromis niloticus* fry production. .Egypt.Ger.Soc.Zool., 43(a): 315-335.

Romerio, M.P.; Fencrich-Verani, C.S.N.; Santo De-Copmus, B.E. and Pasilva, A.S. (2000):

Masculinization of Nile tilapia, using different diets and different doses of MT. Revista Brasileira de Zootecnia, 29: 654-659.

Sambhu, C. and Jayaprakas, V. (1997): Dietary supplementation of testosterone propionate on growth performance of white prawn; *Panaeus indicus* (M. Edwards). Indian J. Experimental. Biol., 35(12): 1353-1358.

Sambrook, L.; Fritsch, E.F. and Manitatis, T. (1989): Molecular cloning: A laboratory manual. Cold Spring Harbor Press, Cold Spring Harbor, N.Y.

Satpathy, B.B.; Mukhopadhyay, P.K. and Ray, A.K. (1995): Nutritive utilization and growth bioenergetics of *Labeo rohita* fry in relation to different dietary protein sources and dietary inclusion of 17 α-methyl testosterone. Pages 262-265. In: M.K. Dasgupta, D.C. Ghosh, D. Das-Gupta, D.K. Majumdar, G.N. Chattopadhyay, P.K. Ganguli, P.S. Munsi and D. Bhattacharya (eds.). Proceedings National Symposium on Sustainable Agriculture in Subumid Zone, March 3-5, Institute of Agriculture, Sriniketan, India.

Saunders, R.L., Fagerlund, U.H.M. and McBride, J.R. (1977): 17 α-methyltestosterone. A potential anabolic hormone in Atlantic salmon culture. Int. Council Explo. Sea, C.M. 1977/E.50, pp 8.

Schreck, C.B. and Moyle, P.B.(1990): Method of fish Biology. American Fisheries Society, Bethesda, Mavyland, USA.

Schwaiger, J.; Fent, K.; Stecher, H.; Ferling, H. and Negele, R. D. (1996): Effect of sublethal concentrations of triphenyltriacetate on rainbow trout (*Oncorchynchus mykiss)*. Arch. Environ. Contam. Toxicol., (30): 327- 334.

Software Program of Statistical Analysis, SPSS. (2008): Software Program of Statistical Analysis, Version 17.0 Edition for Windows. SPSS Inc., Chicago, IL, US.

Statistical Analyses System S.A.S. (2000): Statistical Analyses System, Version 6.2, SAS institution in corporation. Carry, NC27513 USA.

Tayamen, M.M. and Shelton,W.L. (1978): Inducement of sex reversal in *Sarotherodon niloticus* (Linnaeus). Aquacul., 14:349-354.

Teh, S. J.; Adams, S. M. and Hinton, D. E. (1997): Histopathologic biomarker in feral freshwater fish populations exposed to different types of contaminant stress. Aquat. Toxicol., (37): 51- 70.

Weatherley, A.H. and Gill, H.S. (1983): Relative growth of tissues at different somatic growth rates in rainbow trout; *Salmo gairdneri* Richardson. J. Fish Biol., 22: 43-60.

Weatherley, A.H. and Gill, H.S. (1987): Tissues and growth. In "The biology of Fish Growth". St. Edmundsbury Press, Great Britain. Pp. 147- 173.

Williams, J.K.G.; Kubelik, A.R.; Livak, K.J.; Rafalsky, J.A. and Tyngey, S.V. (1990): Molecular changes during gonad masculinization. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., 18: 6531-6535.

Winfree, R.A. and Stickney, R.R.(1981): Effect of dietary protein and energy on growth, feed conversion, feed effiecncy and body composition of tilapia aurea. J.Nutr.III: 1001-1012.

Woo, N.Y.S.,Chung, A.S.B. and Ng, T.B.(1993): Influence of oral administration of 17 β–estradiol and testosterone on growth, digestion, food conversion and metabolism in the underyearling red sea bream, *Chrysophrys* major. Fish Physiol. Biochem., 10(5): 377-387.

Yamazaki, F. (1972): Effects of methyltestosterone on the skin and the gonads of salmonids. Gen. Comp. Endocrinol. Suppl., 3: 741-750.

Yamazaki, F. (1976): Application of hormones in fish culture. J. Fish. Res. Board Can., 33: 948-958.

Zaghloul, K.H.; Omar, W.A. and Abo-Hegab, S. (2005): Environmental hazard risk assessment on *Oreochromis niloticus* and *Tilapia zilli* fsh. J. Egypt. Ger. Soc. Zool., 46(A): 105- 139.